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Ab initio simulations of peptide-mineral interactions

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Abstract

We performed Car-Parrinello Molecular Dynamics (CPMD) simulations of two amino acids, aspartic acid (Asp) and phosphoserine (pSer), on a calcium oxalate monohydrate (COM) surface as a model of the interactions of phosphoproteins with biominerals. In our earlier work using *in vitro* experiments and classical Molecular Dynamics (MD) simulations we have demonstrated the importance of phosphorylation of serine on the interactions of osteopontin (OPN) with COM. We used configurations from our previous classical MD simulations as a starting point for the *ab initio* simulations. In the case of Asp we found that the α -carboxyl and amine groups form temporary close contacts with the surface. For the dipeptide Asp-pSer the carboxyl groups form permanent close contacts with the surface and the distances of its other functional groups do not vary much. We show how the interaction of carboxyl groups with COM crystal is established and confirm the importance of phosphorylation in mediating the interactions between COM surfaces and OPN.

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1. Introduction

Biomineralization is the process that leads to the formation of inorganic-based structures in living organisms [1]. If biologically controlled, organisms regulate mineralization and structures with physiological functions are the end result. Examples of this process are tooth enamel as well as skeletal structures such as bones and shells [1, 2]. Examples of unwanted mineralizations are kidney stones [3] and the calcium mineral deposits involved in atherosclerosis [4].

There are various mechanisms that can control nucleation and crystal growth via molecular-based inhibitors and promoters. Examples of this are proteins such as osteopontin (OPN) and small molecules such as citrate or phosphocitrate. OPN is an acidic, phosphorylated protein found in bone, eggshell and many soft tissues [5]. An upregulation of its gene expression that results in a higher concentration of OPN has been shown in atherosclerotic plaques [6] and kidney stones [7]. It has also been shown that OPN can have an inhibitory effect *in vitro* on the crystal growth of calcium oxalate monohydrate (COM) [8, 9], the main component of kidney stones [3], and hydroxyapatite (HA) [10, 11], which forms the mineral phase in mammalian bone and tooth enamel [2]. Citrate and

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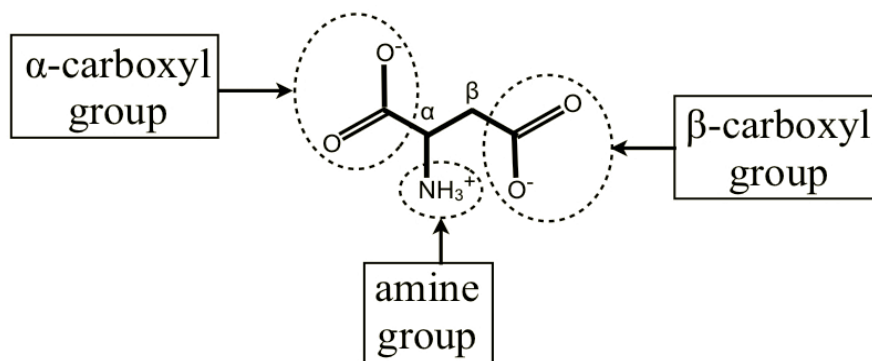


Fig. 1. Chemical structure of Asp showing its functional groups.

phosphocitrate are naturally occurring small molecules for which inhibitory effects on COM nucleation, crystal growth and aggregation, and on HA crystal growth have been shown [12-15]. Understanding the interaction of OPN and other crystallization-inhibiting agents with minerals can lead to the development of new pharmaceutical treatments for diseases involving pathological calcifications. The concepts, molecules and systems involved in biomineralization can also be used in material science to fabricate certain materials, e.g. thin films [16]. Mimicking biomineralization processes offers the possibility to produce materials at low temperatures in an aqueous environment.

The challenge to fully understand the concepts of biomineralization originates from it being a process involving several different time and length scales. The time scales extend from bond vibrations, which are of the order of 10 fs for a heavy-atom-hydrogen bond [17], through nucleation processes taking from seconds to days, to the growth of minerals in the body, which can require years. Bone is an example of a biological material consisting of structures with at least seven different length scales [18]. This range of time and length scales presents a challenge for simulation.

To appropriately capture the important phenomena involved, methods ranging from *ab initio* calculations through classical Molecular Dynamics (MD) simulations to coarse-grained models need to be applied [19, 20]. In the literature, different approaches have been applied to study biomineralization. As an example for the interaction of crystal growth-inhibiting molecules with minerals, the interactions of OPN and citrate with COM and HA have been studied by structure optimization [21, 22] and classical MD simulation [23-28]. Long carboxylic chains or self-assembled monolayers as templates for calcite nucleation [29, 30] and the uptake of carbonate groups in the HA lattice [31] have also been investigated by classical MD. A combination of density functional theory (DFT) calculations with MD has been used to study the uptake of fluoride ions at HA and the hydration of alpha-quartz surfaces [32, 33]. *Ab initio* methods were used to investigate the onset of calcium carbonate nucleation, and the binding of amino acids on HA has also been examined [34, 35].

Classical simulations of the inorganic/organic interface have another kind of challenge due to the fact that force-fields for inorganic and organic materials have been developed independently. To obtain a reliable force-field the organic and inorganic part must be made consistent with each other. This can be very time consuming since such a force-field needs to be validated carefully using, preferably, both experimental data and *ab initio* calculations. A potential model for an organic material interacting with inorganic material has been derived by Ghiringhelli *et al.* for Pt(111) [37] in the OPLS-AA force-field [38]. Another potential model for HA and fluorapatite at the water interface has been derived by de Leeuw [39, 40] using the Born model for solids [41]. In addition to providing a detailed description of the interactions between Asp and Asp-pSer with COM, our simulations also serve as a further first-principles validation of the approach used earlier [23,24].

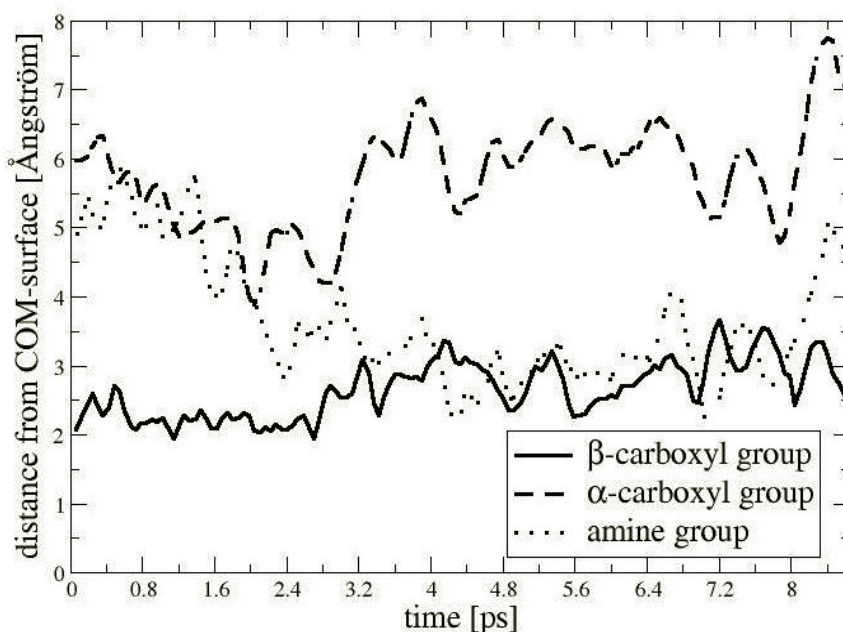


Fig. 2. Distance of the functional groups of Asp from the COM-surface.

We are interested in the inhibition of COM crystal growth by OPN. Understanding this interaction can lead to the development of new treatments for kidney stones and new approaches in material science. In earlier work we performed classical MD simulations of an OPN-related peptide [23]. We used this peptide with different degrees of phosphorylation to investigate the importance of phosphate groups in the interaction of OPN with the {100} face of COM. It was found that amino acids containing carboxyl groups (aspartic acid (Asp) and glutamic acid (Glu)) form the closest contact with the COM surface. It was also found that peptides with a higher degree of phosphorylation bind more strongly, although the phosphates do not seem to interact directly with the crystal surface.

To further investigate the role of phosphate groups in the interaction of OPN with COM, we performed *ab initio* simulations with Asp and the dipeptide of Asp and phosphorylated Serine (pSer). We chose this approach to test and validate the force-field used in the previous classical simulations [23], to determine its ability to accurately model the interactions taking place at this organic/inorganic interface, as well as to gain a deeper understanding of the interactions at the surface. We found that for Asp different functional groups form a temporary close contact to the surface, except for the carboxyl group attached to the β -carbon (β -carboxyl), which stays close during the whole simulation. For the dipeptide, the distance of the functional groups does not vary during the simulation, except that the carboxyl group attached to the α -carbon (α -carboxyl) forms a closer attachment to the surface. This shows that the carboxyl groups are critical for the interaction of OPN with COM, but the role of the other functional groups, especially the phosphate groups, remains to be fully resolved.

2. Computational details

We have performed *ab initio* simulations of systems consisting of the single amino acid Asp, and the dipeptide Asp and pSer (Asp-pSer) on a slab of COM crystal exposing the {100} face to the amino acids. The starting positions for the simulation were taken from the final positions of the previously performed classical MD simulations [23]. The systems were hydrated with 82 water molecules in the Asp-system and 99 molecules in the Asp-pSer-system and the atoms of the crystal were fixed. The cell size was 1.4 nm x 2.2 nm x 1.63 nm for Asp and 1.5 nm x 2.243 nm x 1.878 nm for Asp-pSer. The normal of the crystal surface is pointing in the y-direction. Periodic boundary conditions were applied in all directions. The simulations were performed using the CPMD [42] package version

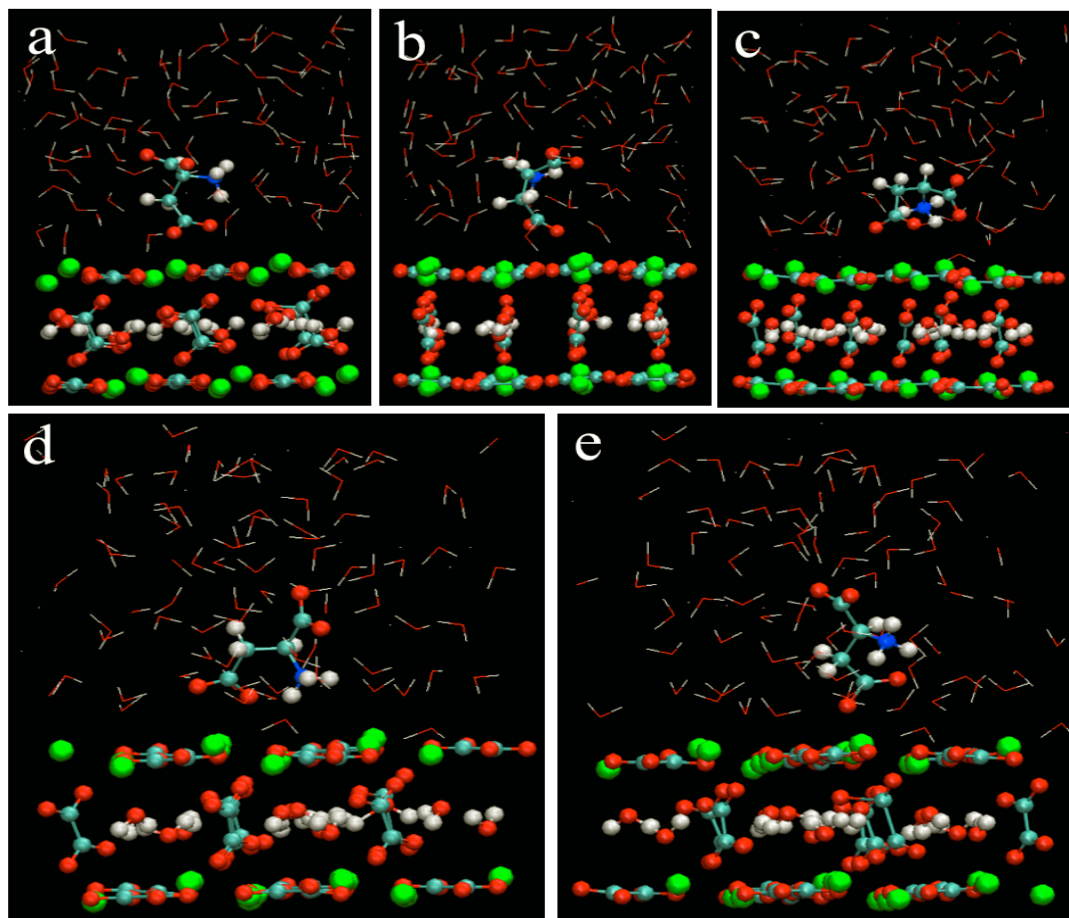


Fig. 3. (a) and (b) snapshots of start configuration from different angles. (c) snapshot at 2.7 ps: all the functional groups are pointing towards surface. (d) snapshot at 4.9 ps: just one β -carboxyl group and the α -amine group are pointing towards the surface. (e) snapshot at 8.5 ps (end of simulation): only the β -carboxyl group is pointing towards the surface. O – red, H – white, N – blue, C – turquoise, Ca – green.

3.13.2 in the NVT ensemble at 300K using the Nosé-Hoover thermostat [43-47]. The time step for the integration was set to 5 a.u. (≈ 0.12 fs). The Ewald method [36] was used to compute electrostatic interactions. The fictitious mass was 800 a.u. and the plane waves were cut off at 70 Ry. This cut-off was chosen similarly with other work done in this field [48, 49]. Martin-Troullier pseudopotentials and BLYP DFT exchange correlation functional [50, 51] were used. The simulation time was 8.64 ps for the system with Asp and 3.4 ps for Asp-pSer. The simulations were run in parallel over 32 or 64 processors on the SHARCNET grid computing facility (www.sharcnet.ca). In total, the simulations took about 120 000 CPU hours.

3. Results

3.1 Asp on {100} COM

The functional groups of interest in Asp are the α - and β -carboxyl groups as well as the amine group as shown in Figure 1.

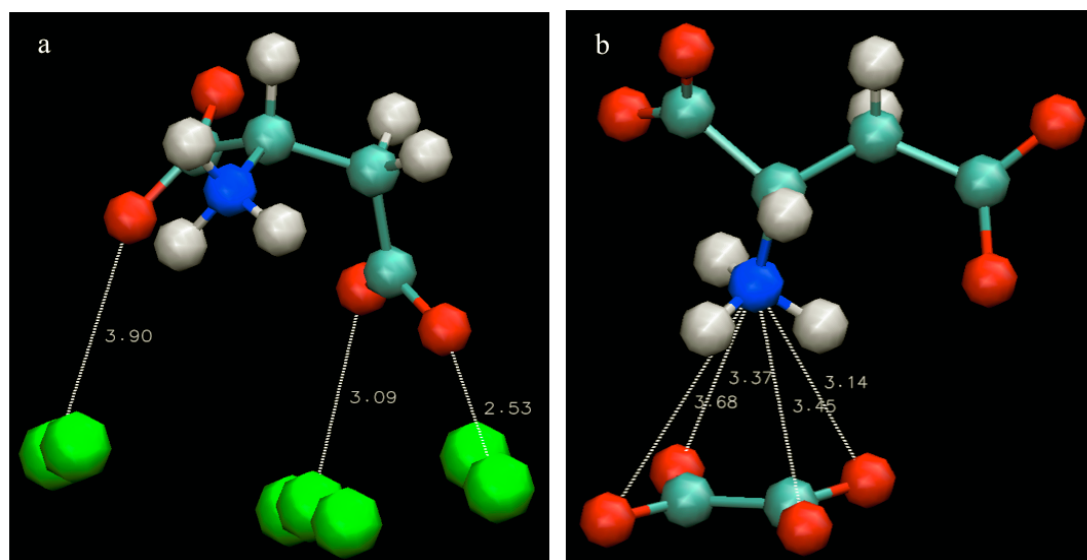


Fig. 4. Interaction of the oxygen of the carboxyl groups of Asp and the calcium ions of COM (snapshot at 4.14 ps): three oxygen form a relatively close contact to the calcium. (b) Interaction of the amine groups of Asp with the oxalate of COM (snapshot at 4.14 ps). O – red, H – white, N – blue, C – turquoise, Ca – green. All distances in Å.

Figure 2 shows the distances of the different functional groups of Asp from the crystal surface. From this graph it can be seen that the β -carboxyl group, which was close to the surface at the starting position, stays relatively close to the surface during the whole simulation time. The α -carboxyl group is moving towards the surface at the beginning of the simulation and then moving away again. The α -amine group forms a closer contact with the crystal surface during the simulation, but at the end of the simulation, the distance increases again.

Figure 3 (a) and (b) show snapshots of the starting position of the system perpendicular to and along the crystallographic c axis, respectively. Figure 3 (c) shows a snapshot at 2.7 ps, where all the functional groups are pointing towards the surface. Figure 3 (d) shows a snapshot at 4.9 ps, where just the β -carboxyl group and the α -amine group are pointing towards the surface. Figure 3 (e) shows a snapshot towards the end of simulation, where just the β -carboxyl group is interacting with the surface, just as at the beginning of the simulation.

Figure 4 (a) shows the closest interaction of the oxygen of Asp with the calcium ions of COM. The oxygen of the β -carboxyl group forms a close contact (bond length between 2.5 and 3 Å) with the calcium ions of COM between 0.66 ps and 2.7 ps. The α -carboxyl group forms a short contact with one oxygen atom, but never as close as the β -carboxyl group. Figure 4 (b) shows the closest interaction of the amine of Asp with an oxalate of COM. For 3.9 ps (from 2.4 ps to 6.3 ps) the bond distances between the nitrogen and the oxygen atoms are in the range of 3.5–4.5 Å.

We did not observe permanent contact between any of Asp's functional groups and the COM surface in the simulations using Asp. This observation is consistent with the earlier classical MD simulations [23]. Next we analyze the system with Ser phosphorylated.

3.2 Asp-pSer on {100} COM

In our previous classical MD simulations [23] we found that amino acids containing carboxyl groups (Asp and Glu) form the closest contact with the COM-surface and that peptides with a higher degree of phosphorylation bind more strongly. For this reason we have chosen to simulate the dipeptide Asp-pSer in comparison with Asp to study the effect of a phosphate group.

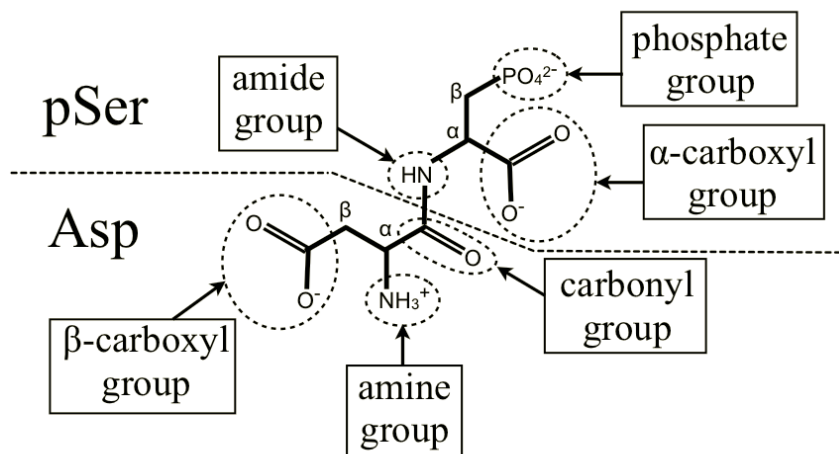


Fig. 5. Chemical structure of Asp-pSer showing its functional groups.

The interesting functional groups of the Asp-pSer (Fig. 5) are the β -carboxyl group, the carbonyl group and the amine group of Asp and the α -carboxyl group, the phosphate group and the amide group of pSer.

Figure 6 shows a plot of the distances of these functional groups from the COM surface. It can be seen that there is no drastic change in the distance for any functional group during the simulation time, except the α -carboxyl group which forms a closer contact with the surface.

Figure 7 (a) and (b) show snapshots of the starting position of the system along the crystallographic b and c axes, respectively, where the β -carboxyl group, the carbonyl group and the amine of Asp and the α -carboxyl group of pSer are interacting with the surface. Figure 7 (c) shows a snapshot at the end of the simulation, where the direction of the functional groups is basically the same as at the beginning of the simulation. Only the amine group seems to interact less with the surface than at the beginning of the simulation.

The interaction between the carboxyl group of Asp-pSer and COM is formed through a bond between the two oxygen of the carboxyl group and two neighboring calcium ions of COM. The bond length is approximately 2.45 Å, the same as between the oxygen in the oxalate and the calcium ions in the COM crystal. First, at 0.54 ps the oxygen of the β -carboxyl of Asp-pSer forms a close and stable contact with the calcium. The α -carboxyl group of Asp-pSer first interacts with one oxygen atom and later the second oxygen follows. After 1.62 ps the first oxygen atom of the carboxyl group remains at a distance shorter than 2.5 Å and the second smaller than 3.5 Å. Figure 8 shows the interaction between the carboxyl groups of Asp-pSer and the calcium ion of COM-surface at the end of the simulation, where the distances reach their minimum.

4. Conclusions

Our previous classical MD simulations [23] demonstrated that amino acids containing carboxyl groups (Asp and Glu) form the closest contact with the COM surface and that peptides with a higher degree of phosphorylation bind more strongly. These results show that in the interaction of a protein with a COM crystal the crucial components are acidic and phosphorylated amino acids. Here, we report results from *ab initio* simulations of Asp and Asp-pSer on the {100} face of COM to investigate the importance of the phosphate group on the interactions. We measured the distance of the functional groups to the crystal surface as a function of time and analyzed the various contacts and

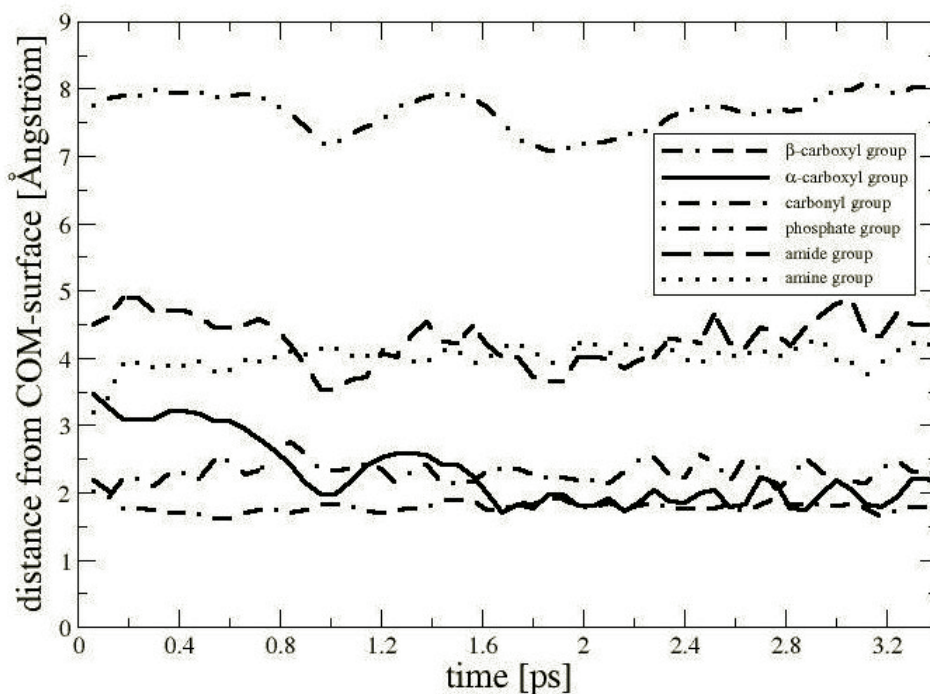


Fig. 6. Distance of the functional groups of Asp-pSer from the COM-surface.

their lifetimes. We found that for Asp, the β -carboxyl and the amine groups interact with the surface but neither forms a stable contact during the simulations. The most prominent interaction for the dipeptide is between the carboxyl groups of Asp-pSer and the calcium ions of COM. During the simulation, the amino acids can be observed forming a gradual attachment with their carboxyl oxygens. Once an interaction is formed it remains stable during the whole simulation.

These results confirm the findings of *in vitro* experiments and classical MD simulations performed earlier [23, 24, 52] in showing the strong effect of the presence of a phosphate group on the attachment of the carboxyl groups with

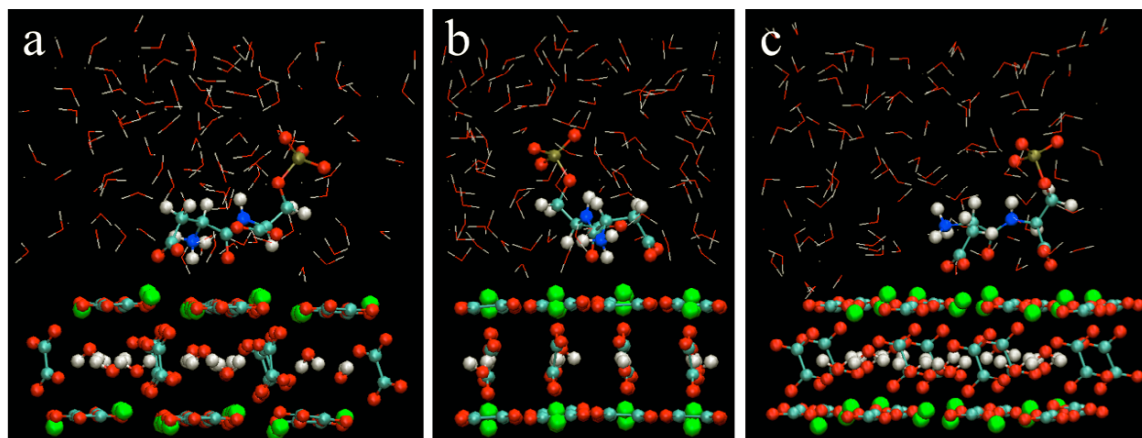


Fig. 7. (a) and (b) snapshots of start configuration from different angles. (c) snapshot at 3.4 ps (end of simulation): All the functional groups containing oxygen, except the phosphate group, are pointing towards the surface.

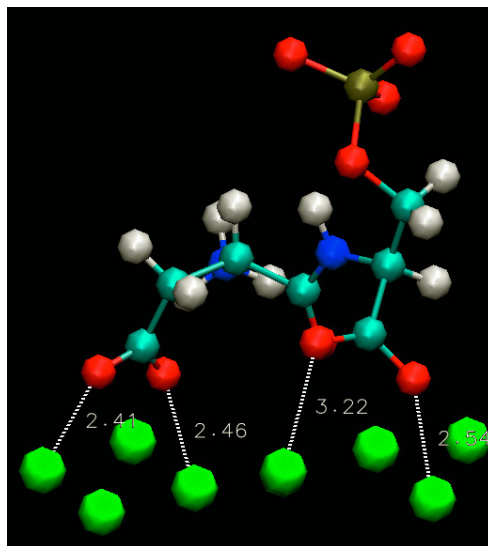


Fig. 8. Interaction of the carboxyl groups of Asp-pSer with the calcium ions of COM (snapshot at 3.36 ps): both carboxyl group form a close contact to the calcium ions. O – red, H – white, P – brown, N – blue, C – turquoise, Ca – green. All distances in Ångströms.

the crystal surface. In the system without phosphate, the carboxyl groups do not form a permanent attachment to the COM surface, as they do in the system with the phosphate, although the simulation time for the latter is even shorter. The distance of a carboxyl group of an amino acid interacting with the COM surface has been found to be around 2 Å in our *ab initio* calculations. This is in agreement with the closest distance of acidic residues in the classical MD simulations we have performed earlier [23]. Although the CPMD simulations were performed with amino acids instead of the longer peptides and the simulation time was much shorter than the classical MD simulations, we see a correlation between the two methods of simulations supporting the results of the classical MD simulations.

Compared to other CPMD simulations which consist of 100–200 atoms, our systems are very large, containing 432 and 527 atoms for Asp and Asp-pSer, respectively. For this reason, the simulation time used in our studies is somewhat lower than in some other reported simulations (up to 24 ps for smaller systems). We used, however, configurations from our previous 50-ns MD simulations as a starting point in order to have an equilibrated starting configuration. In our simulations it has also been possible to analyze the behavior of the system at equilibrium and calculate quantitative properties. Thus we have been able to elucidate part of the mechanism of the interaction of carboxyl groups with the COM crystal and confirm the importance of the presence of phosphate groups in mediating the close interactions with crystal surfaces found in experiments and simulations [23, 24, 52].

Acknowledgements

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